REMARKS

Claims 1-18 and 33-47 are pending; claims 2-4, 6, 8-18, and 33-47 are withdrawn from consideration. Claims 1, 5, and 7 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. Claims 1, 5, and 7 are rejected under 35 U.S.C. § 112, second paragraph, for indefiniteness. Claims 1, 5, and 7 are rejected under 35 U.S.C. § 102(b) for anticipation by Shapiro et al. (New England J. Med. 243:230-238, 2000; hereinafter "Shapiro"). Finally, the Office requests a listing of all copending U.S. applications that set forth similar subject matter to the present claims and that share an inventor or assignee in common with the instant application. By this reply, Applicant cancels claims 2-6, 8-18, and 33-47, amends claims 1 and 7, adds new claims 48-54, and addresses each of the Office's rejections.

Support for the Amendment

Support for the amendment to claims 1 and 7 is found in the specification at, e.g., page 11, line 26, through page 12, line 9, and page 16, lines 4-20. Support for new claims 48-54 is found in the specification at, e.g., page 22, lines 8-20, and prior claims 18 and 33. No new matter is added by the amendment.

Rejections under 35 U.S.C. § 112, second paragraph

The Office rejects claims 1, 5, and 7 under 35 U.S.C. § 112, second paragraph, for indefiniteness, stating:

> Claim 1 is drawn to "a method of treating a pancreatic disease ... comprising the step of administering to a patient [a cell]," which is confusing because the claim does not particularly require that the patient being administered the cell be afflicted with the disease. Clarification is required.

(Office Action, p. 7.) Applicant has amended independent claim 1 to recite a method of treating diabetes in a patient in need thereof. This rejection can be withdrawn.

The Examiner also states that the phrase "a pluripotent cell" in claim 1 is not adequately defined, stating:

> The specification equates [this phrase] with "stem cell," which includes both pluripotent ES cells and multipotent mesenchymal stem cells (i.e., the cells with the expression pattern in elements (a) and (b) of claim 1); see page 6. However, the specification also refers to "a number of types of mammalian pluripotent cells," including among them multipotent mesenchymal stem cells and pluripotent ES cells. See page 1, lines 10-25. It is not clear whether applicant intends "pluripotent" to have its accepted meaning or the contrary one. Clarification is required.

(Office Action, pp. 8-9.)

The present specification defines the phrase "pluripotent cell" at page 6, lines 13-15, as "a cell having the ability to give rise to two or more cell types of an organism." This definition is not unclear, nor is it inconsistent with its usage in the art. Furthermore, the cell recited in present independent claim 1, and claims dependent therefrom is prepared from umbilical cord blood or placental blood, and thus it is not an embryonic stem cell, as that phrase is known and used in the art. Nonetheless, to avoid confusion, Applicant has amended claim 1 to remove the term "pluripotent." This rejection can be withdrawn.

The Office also rejects claim 1, stating that the term "a progeny cell derived [from a pluripotent cell]...is indefinite" (Office Action, p. 9). Applicant has amended claim 1 to remove the phrase "a progeny cell derived therefrom." This rejection can be withdrawn.

The Office rejects claim 1 stating that "[c]laim 1 requires that the cell be 'capable of' differentiating into any of various cell types, but it is not clear whether this differentiation is necessarily part of the claimed method or whether it merely describes an ability that the cells have under some unnamed conditions" (Office Action, p. 9). Applicant has amended claim 1 to remove this phrase. This rejection can be withdrawn.

Finally, the Office rejects claim 7, stating:

> Claim 7 requires that the method comprise "administering said cell to effect organ regeneration," but it is not clear whether this regeneration actually occurs as part of the method or whether this limitation merely recites one possible intended use of the administration step. Clarification is required.

(Office Action, p. 9.) Applicant has amended claim 7 to recite that the method involves "administering said cell to effect regeneration of pancreatic islet cells." The action of the administered cell may be direct (e.g., the cell may differentiate into pancreatic islet cells) or indirect (e.g., the cell may slow the decline of pancreatic islet cells by modulating the immune response). This rejection should be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

> The Office rejects claims 1, 5, and 7, stating:
>
> the specification, while being enabling for treating a pancreatic disease by administering a progeny cell that is a pancreatic cell to a patient with a pancreatic disease, does not reasonably provide enablement for treating any pancreatic disease by administering the pluripotent cell described in claim 1 or by administering any non-pancreatic progeny cell. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

(Office Action, pp. 2-3.) Applicant has amended independent claim 1 to recite a method for treating diabetes in a patient in need thereof by administering a cell prepared from human umbilical cord blood or placental blood, in which the cell (a) expresses the SH2, SH3, SH4, CD13, CD29, CD49e, CD54, and CD90 antigen markers and (b) does not express the CD14, CD31, CD34, CD45, CD49d, or CD106 antigen marker. Thus, present claim 1 is directed to the treatment of diabetes rather than any pancreatic disease.

The specification provides considerable guidance with respect to the cells to be administered and how to obtain those cells. As is discussed above, cells to be administered are designated as positive for the SH2, SH3, SH4, CD13, CD29, CD49e, CD54, and CD90 antigen markers and negative for the CD14, CD31, CD34, CD45, CD49d, or CD106 antigen marker and can be prepared from umbilical cord blood or placental blood. In addition, the specification teaches formulations for administering the cells and how to administer the cells (see, e.g., page 9, line 6, through page 14, line 28, page 16, lines 4-25, and page 22, line 24, through page 23, line 9).

Finally, Applicant directs the Office to Haller et al. (Diabetes Care 32:2041-2046, 2009) as evidence that cord blood cells can be safely administered to young diabetic subjects and that "subjects receiving umbilical cord blood infusion maintained A1C and insulin requirements below what most clinicians would expect in such young children...[, although] among umbilical cord blood recipients, peak and area under the curve C-peptide levels 1 year post–umbilical cord blood infusion declined significantly when compared with baseline and fractional changes in A1C, and insulin requirement were no different when comparing umbilical cord blood recipients and historical control subjects" (see page 5; see also Haller et al. (Exp. Hematol. 36:710-715, 2008); copies of both publications are provided).

For all the reasons discussed above, Applicant's specification enables the administration of the claimed cells to treat diabetes in a patient in need thereof. This rejection should be withdrawn.

Rejections under 35 U.S.C. § 102(b)

The Office rejects claims 1, 5, and 7 under 35 U.S.C. § 102(a) for anticipation by Shapiro, stating:

> The limitation "a progeny cell derived [from a pluripotent cell having certain properties is a product-by-process limitation...[I]slets obtained directed from a pancreas (as were Shapiro's) will be considered identical to islets derived from the pluripotent cells recited in claim 1"

(Office Action, pp. 10-12.)

To form the basis of a proper rejection under 35 U.S.C. § 102, a cited reference must disclose each and every element of the rejected claim. *See Lewmar Marine Inc. v. Barient Inc.*, 3 U.S.P.Q.2d 1766 (Fed. Cir. 1987) and Manual of Patent Examining Procedure (MPEP) § 2131.

Applicant has amended independent claim 1 to remove the phrase "a progeny cell derived [from a pluripotent cells]." As is acknowledged by the Office, Shapiro only describes the administration of pancreatic islet cells (Office Action, p. 10). Shapiro fails

to disclose the administration of a cell that is positive for the SH2, SH3, SH4, CD13, CD29, CD49e, CD54, and CD90 antigen markers and negative for the CD14, CD31, CD34, CD45, CD49d, or CD106 antigen marker, as is recited in present independent claim 1, and claims dependent therefrom. Thus, Shapiro fails to disclose each and every element of present claim 1, 7, and 48-54. This rejection should be withdrawn.

Listing of Related U.S. Applications

The Office requests Applicant to provide a list of all copending U.S. applications that set forth similar subject matter to the present claims and share an inventor or assignee with the instant application. As requested, Applicant lists the following related U.S. patent and application:

- U.S. Patent No. 7,560,280
- U.S. Patent Application Publication No. 2009/0238803

CONCLUSION

Applicant submits that present claims 1, 7, and 48-54 are in condition for allowance, and such action is respectfully requested.

Transmitted herewith is a petition to extend the period for replying for three months, to and including November 15, 2010, and authorization to deduct the fee required under 37 C.F.R. § 1.17(a) from Deposit Account No. 03-2095. If there are any other charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 15 November 2010

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Autologous Umbilical Cord Blood Transfusion in Very Young Children With Type 1 Diabetes

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Abstract

OBJECTIVE Interest continues to grow regarding the therapeutic potential for umbilical cord blood therapies to modulate autoimmune disease. We conducted an open-label phase 1 study using autologous umbilical cord blood infusion to ameliorate type 1 diabetes.

RESEARCH DESIGN AND METHODS Fifteen patients diagnosed with type 1 diabetes and for whom autologous umbilical cord blood was stored underwent a single intravenous infusion of autologous cells and completed 1 year of postinfusion follow-up. Intensive insulin regimens were used to optimize glycemic control. Metabolic and immunologic assessments were performed before infusion and at established time periods thereafter.

RESULTS Median (interquartile range [IQR]) age at infusion was 5.25 (3.1–7.3) years, with a median postdiagnosis time to infusion of 17.7 (10.9–26.5) weeks. No infusion-related adverse events were observed. Metabolic indexes 1 year postinfusion were peak C-peptide median 0.50 ng/ml (IQR 0.26–1.30), $P = 0.002$; A1C 7.0% (IQR 6.5–7.7), $P = 0.97$; and insulin dose 0.67 units · kg^{-1} · day^{-1} (IQR 0.55–0.77), $P = 0.009$. One year postinfusion, no changes were observed in autoantibody titers, regulatory T-cell numbers, CD4-to-CD8 ratio, or other T-cell phenotypes.

CONCLUSIONS Autologous umbilical cord blood transfusion in children with type 1 diabetes is safe but has yet to demonstrate efficacy in preserving C-peptide. Larger randomized studies as well as 2-year postinfusion follow-up of this cohort are needed to determine whether autologous cord blood-based approaches can be used to slow the decline of endogenous insulin production in children with type 1 diabetes.

Type 1 diabetes is an autoimmune disorder characterized by T-cell-mediated destruction of insulin-producing β-cells and lifelong dependence on exogenous insulin administration. To date, the majority of efforts seeking to ameliorate the autoimmune process and reverse hyperglycemia have focused on the use of immunosuppressive or immunomodulatory drugs (1–4). Although several agents have shown and continue to show promise, no single agent has succeeded in demonstrating long-term success in preventing or reversing type 1 diabetes as a means of standard medical practice. More recently, efforts have focused on the use of either autologous or allogeneic hematopoietic stem/progenitor cells as potential immunoregulatory agents to reverse this disease. Whereas hematopoietic stem cells have successfully been directed in vitro to differentiate into insulin- and C-peptide-producing cells (5), and infusion of human hematopoietic stem cells into diabetic animals has demonstrated reversal of disease (6,7), the potential of such cells to provide a source of safe and effective immunomodulation may be of the greatest importance in treating type 1 diabetes, but this has yet to be realized (8–10).

Among the broad array of potential cell-based therapies, the use of autologous umbilical cord blood as a source of immunomodulatory cells for the treatment of autoimmune diseases has become increasingly popular (11–14), this based on the potential for umbilical cord blood to restore proper immune regulation. Umbilical cord blood contains a robust population of immature unprimed highly functional regulatory T-cells (Tregs) (15). These highly functional Tregs could, in theory, limit inflammatory cytokine responses and anergize effector T-cells, which are thought to play a key role in cellular-mediated autoimmune processes (16,17). As such,

umbilical cord blood Tregs have become a major focus of our work in designing cell-based therapies for children with type 1 diabetes (18).

Practical matters provide an additional rationale for umbilical cord blood-based therapies. First, the lack of low-risk (i.e., safe) diabetes intervention trials seeking to reverse disease, especially for young children with type 1 diabetes, renders the potential use of umbilical cord blood particularly appealing. Second, as the rates of umbilical cord blood storage continue to increase exponentially, the number of potential subjects for autologous umbilical cord blood-based clinical trials continues to grow. Third, the fact that umbilical cord blood is stored at birth without need for additional intervention (i.e., bone marrow biopsy or stem cell mobilization and aphaeresis) is an additional practical advantage in considering a cell-based therapy for children. Finally, as umbilical cord blood storage facilities continue to reevaluate storage methods that would allow for multiple withdrawals, potential exists for protocols that involve cell expansion and/or multiple cell infusions.

Although we focused our interest on the notion that umbilical cord blood Tregs might affect tolerance, we also considered that autologous umbilical cord blood transfusion in the setting of type 1 diabetes may help mitigate the autoimmune process by a variety of mechanisms beyond those of direct immune modulation (19). First, umbilical cord blood stem cells may migrate to the damaged pancreas, where they could differentiate into insulin-producing β-cells (2). In addition, umbilical cord blood stem cells might act as nurse cells to foster the proliferation or replication of new β-cells from remnant viable tissue (20). Finally, umbilical cord blood Tregs may facilitate bystander suppression of effector T-cells, allowing for the restoration of tolerance by their inhibitory effects on multiple cell types (21).

Based on available preclinical data and the agreement that infusion of minimally manipulated autologous umbilical cord blood was likely to be extremely safe, we performed an unblinded observational pilot study to determine whether autologous umbilical cord blood infusion could impede the type 1 diabetes autoimmune process and preserve remaining endogenous insulin production. Peak C-peptide after a standard mixed-meal tolerance test (MMTT), A1C, and daily insulin requirement were set as the primary outcome variables, with a variety of immunologic markers assessed for their potential mechanistic insights.

RESEARCH DESIGN AND METHODS

A detailed description of the study protocol's design, without results, has been published previously (18). The study timeline is displayed in Fig. 1. Briefly, subjects aged >1 year with type 1 diabetes (established by clinical presentation and presence of type 1 diabetes-associated autoantibodies) and for whom autologous umbilical cord blood had been stored in an American Association of Blood Banks (AABB)- or Foundation for the Accreditation for Cellular Therapy (FACT)-recognized cord blood bank, were recruited for participation in this single-center study (NCT00305344; FDA IND BB-11918). This Federal Drug Administration (FDA)-approved study of 23 subjects completed enrolment in November 2008. Follow-up for the entire cohort will continue until all subjects have reached the 2-year postinfusion study visit. For this report, the first 15 of these 23 subjects who completed at least 1 year postinfusion follow-up are reported herein. In addition, because the FDA and institutional review board did not allow, on this occasion, for the implementation of a placebo-controlled investigation, for comparative purposes we collected retrospective data on type 1 diabetic subjects matched 2:1 (control subjects to umbilical cord blood recipients) for age and duration of disease.

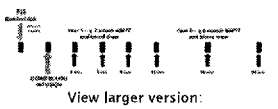

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Figure 1

Autologous cord blood infusion in type 1 diabetes: study timeline. Our study was designed as a 2-year observational study of the effects of autologous cord blood infusion in children with type 1 diabetes. Each child was followed every 3 months during the first year postinfusion and every 6 months during the second year postinfusion. Blood was obtained for metabolic and immunologic studies at each visit. Mean time from type 1 diabetes

diagnosis to umbilical cord blood infusion was 6 months. Herein, we report 1 year postinfusion data on the first 15 umbilical cord blood recipients to reach 1 year of post-cord blood infusion follow-up. T1D, type 1 diabetes; UCB, umbilical cord blood; q, every.

After eligible subjects were identified and provided consent, peripheral blood and an aliquot of umbilical cord blood from the subject were shipped to the University of Florida stem cell laboratory where infectious disease testing, HLA confirmation, and screening for viability were performed. Thereafter, the subject's remaining umbilical cord blood unit was shipped to the University of Florida and stored until transfused. Subjects were then scheduled to perform a standard 2-h MMTT to determine baseline endogenous insulin production and A1C values. Additional blood was drawn for routine clinical assessments as well as metabolic and immunologic analyses.

On the subsequent day, the subject's autologous umbilical cord blood was thawed and washed per standard operation procedures of the University of Florida stem cell laboratory. An aliquot of cells was analyzed for viability, CD34 percentage, and Treg frequency (i.e., percentage of CD3+CD4+CD25+FOXP3+ cells). After the preparation of the unit, subjects received pretreatment with diphenhydramine and acetaminophen. No chemotherapy or other preparative therapy was given. The thawed umbilical cord blood cells (typically <100 ml) were then infused through a peripheral intravenous drip over 10–20 min. After infusion, subjects were observed closely for at least 6 h prior to being discharged.

Subjects returned for follow-up testing every 3 months in the first postinfusion year and every 6 months in the second postinfusion year. MMTT, complete blood count, basic metabolic panel, and A1C were performed at each visit. In addition, whole blood collected in EDTA tubes was analyzed by flow cytometry with staining for the cell surface markers CD3, CD4, CD8, and CD25 as well as the intracellular marker FOXP3 using standard techniques (21–23). Throughout the study, subjects were encouraged to use any combination of available intensive insulin regimens to achieve the best possible glycemic control. Use of hypoglycemic agents other than insulin or any immunosuppressive agents was not permitted.

Statistical analysis

Because of the propensity for outliers in several of the outcome variables, medians and quartiles were reported rather than means and SDs. To determine changes from baseline to 12 months, we calculated the fractional change for each subject as $[(Y_{12}/Y_0) - 1]$, where the subscript is the month number. These fractional changes were then tested for a target population null hypothesis of a median of zero by the two-sided Wilcoxon sign-rank test, a nonparametric procedure. The pilot nature of this study dictated against controlling study-wide errors via either a Bonferroni correction or formal multivariate analysis. Historical control comparisons used the two-sided two-sample Wilcoxon test.

RESULTS

Between 24 August 2005 and 21 November 2008, 23 children with type 1 diabetes (10 male and 13 female) underwent a single autologous umbilical cord blood transfusion at the University of Florida. As of January 2009, 15 subjects (7 male and 8 female) completed 1 year of postinfusion study follow-up. Baseline and 1-year postinfusion characteristics of these 15 subjects are provided in Table 1.

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Table 1

Baseline and 1-year postinfusion characteristics of autologous umbilical cord blood recipients (n = 8 male and 7 female)

With the exception of one autologous umbilical cord blood unit recovered from a public bank, the remaining 22 units were stored in private cord blood facilities throughout the U.S., Canada, and Mexico. The median infused total nucleated cell count in those subjects with 1 year of follow-up was 1.50×10^7 cells/kg. Median viability was 96% (range 92–99%). Overall, the total nucleated cell count recovered was commonly 1- to 2-log-fold less than that typically observed in samples obtained from public banks (24).

All aliquots of umbilical cord blood had negative gram stains, and none grew pathogenic organisms when cultured for virus, bacteria, or fungus. No adverse events were observed in association with autologous umbilical cord blood infusion. None of the 23 subjects receiving the cellular infusion developed fever, hypo- or hypertension, nausea or vomiting, abnormalities in serum creatinine, or clinically relevant changes in complete blood count parameters. Furthermore, no subject reported a severe hypoglycemic event (seizure or hypoglycemia requiring assistance) or admission for treatment of diabetic ketoacidosis throughout the first year of follow-up.

In the 15 subjects with at least 1 year of study follow-up (average 18 months postdiagnosis), median peak C-peptide (interquartile range [IQR]) at the time of autologous umbilical cord blood infusion was 0.93 (0.7–2.03 ng/ml). At the 1 year post-umbilical cord blood infusion visit, median peak C-peptide was 0.5 (0.26–1.30 ng/ml). The fractional change in peak C-peptide from infusion to 1 year was −53% ($P = 0.0024$). The fractional change of area under the curve C-peptide over the 1 year after umbilical cord blood infusion was −69.4% ($P = 0.0007$). For the same group of study subjects, initial median A1C was 7.0% and 1 year post-umbilical cord blood infusion A1C was 7.0%. Median fractional change in A1C was 0.035% ($P = 0.45$). Initial median insulin requirement was 0.42 units · kg^{-1} · day^{-1} with 1 year post-umbilical cord blood insulin requirements of 0.67 units · kg^{-1} · day^{-1}. The median fractional increase in insulin requirement over 1 year was 52% ($P = 0.0085$).

In order to further identify a potential clinical benefit for this procedure, a comparative analysis of A1C and insulin use in this group of umbilical cord blood-treated subjects versus a historical control group of 30 type 1 diabetic patients was also performed. Because the average time from type 1 diabetes diagnosis to umbilical cord blood infusion was 6 months, comparisons were made between umbilical cord blood recipients at 0, 6, and 12 months postinfusion and control subjects at 6, 12, and 18 months post-type 1 diabetes diagnosis. In this group of control subjects (mean age 5.28 years at diagnosis), median A1C and insulin use 18 months after diagnosis were 7.8 and 0.77% units · kg^{-1} · day^{-1}, respectively. Umbilical cord blood recipients demonstrated lower preinfusion insulin requirements ($P = 0.011$) but similar A1C ($P = 0.16$) when compared with the historical control subjects. Whereas the A1C was, as noted earlier, stable over the 1 year of follow-up in umbilical cord blood recipients, the percent change in A1C and insulin use were not significantly different when comparing umbilical cord blood recipients and control subjects at the 12-month follow-up visit (Table 2, Fig. 2*A* and *B*). Fractional changes in A1C and insulin requirement at 6 months postinfusion were also not significantly different between umbilical cord blood recipients and historical control subjects (data not shown).

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Table 2

Historical control subjects (n = 30)

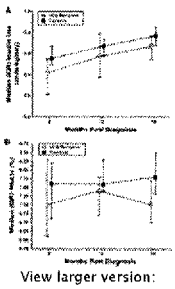

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Figure 2

Insulin use (*A*) and A1C (*B*) in umbilical cord blood recipients and historical control subjects over 1 year. Data are medians with error bars showing IQRs and are presented for the sake of comparison with clinical expectations. Because the average time from diagnosis to umbilical cord blood infusion was 6 months, comparisons correlate with 6, 12, and 18 months post-type 1 diabetes diagnosis in both umbilical cord blood recipients and historical control subjects. Whereas the absolute value of A1C and insulin requirement in umbilical cord blood recipients was lower than that in control subjects at baseline, 6 months, and 12 months, the relevant statistical

comparison of percent change was no different over time (Table 2).

Primary analyses examined changes in serum and peripheral blood immune markers from cord blood infusion to the 1 year postinfusion visit. Baseline and 12-month median total peripheral white blood cell count were 5.5×10^9 cells/l and 4.9×10^9 cells/l, respectively, indicative of a −12.9 fractional change ($P = 0.03$). Median and 12-month post-infusion serum GAD antibody concentrations were 4.2 units/ml and 3.5 units/ml, respectively ($P = 0.52$). Serum insulinoma-associated protein 2 autoantibody at infusion and 12 months later were unchanged at 9.5 units/ml and 9.8 units/ml, respectively ($P = 0.20$). CD4-to-CD8 ratio was 1.98 at screening and 1.93 12 months after cord blood infusion ($P = 0.85$). $CD4^+CD25^+FOXP3^+$ (Treg) percentages in peripheral blood at infusion and after 12 months were 5.4 and 5.0%, respectively ($P = 0.12$) (Table 1). No changes in peripheral blood CD45RA (naïve) or CD45RO (memory) cells were observed when comparing baseline and 12-month postinfusion data (data not shown). Hypothesis-generating analysis of the changes in peripheral blood Treg concentration demonstrated a 42% fractional increase of Treg during the 6 months immediately after cord blood infusion ($P = 0.06$) with the majority of that increase occurring between the 3 and 6 months visits (fractional increase in Treg 35.6%; $P = 0.01$). No changes in CD4-to-CD8 ratio, CD45RA, or CD45RO cells were seen when comparing data at infusion, 3 months, or 6 months.

CONCLUSIONS

As the first study of a cell-based therapy in children with type 1 diabetes, the most important and robust observation our phase I study provides is that autologous umbilical cord blood infusion in young children with type 1 diabetes is feasible and safe. However, the potential efficacy of autologous umbilical cord blood infusion in the type 1 diabetes setting remains less clear. The currently available data suggest that autologous umbilical cord blood infusion fails to preserve C-peptide levels in young children 1 year after infusion. As such, the 2 years postinfusion data for the entire 23-subject cohort of umbilical cord blood recipients will be important in more conclusively documenting the efficacy of autologous umbilical cord blood infusion in type 1 diabetes.

Because neither the FDA nor our local institutional review board would allow for a randomized or blinded study, we were unable to perform age-matched comparisons of meal-stimulated endogenous insulin production. Furthermore, the lack of comparative meal-stimulated C-peptide data in young children with type 1 diabetes made historical comparisons impossible. Comparison to an age-matched group of type 1 diabetic patients demonstrated that subjects receiving umbilical cord blood infusion maintained A1C and insulin requirements below what most clinicians would expect in such young children. Nevertheless, among umbilical cord blood recipients, peak and area under the curve C-peptide levels 1 year post-umbilical cord blood infusion declined significantly when compared with baseline and fractional changes in A1C, and insulin requirement were no different when comparing umbilical cord blood recipients and historical control subjects.

As we further explore potential applications of autologous umbilical cord blood in treating type 1 diabetes, practical considerations will continue to drive our approach. Notably, the cell counts recovered from privately banked cord blood units used in our study were routinely an order of magnitude lower than cell counts from publicly banked units prepared using the same techniques. This should not necessarily impugn private cord blood banks for providing substandard storage but more likely reflects the frequency of low cell counts at collection and explains why public banks collect and then discard a large percentage of donated units. The relatively low cell counts used in this phase I study may indicate that much higher cell counts are needed to induce relevant immunologic or metabolic effects. Additional efforts to improve collection, storage (i.e., multiple aliquots), cell recovery, and expansion of umbilical cord blood are urgently needed to allow for the development of additional applications beyond traditional umbilical cord blood transplantation.

Additionally, the dictum of primum non nocere must remain paramount when discussing interventional therapies for young children with type 1 diabetes. The development of both safe and effective therapies to preserve β-cell function in patients with type 1 diabetes presents a formidable catch-22. Although high-potency immunosuppressive and immunomodulatory cocktails may indeed be able to preserve C-peptide levels in recently diagnosed patients (14), such approaches

are associated with considerable risk of morbidity and even mortality. Type 1 diabetes is inarguably a disease with substantial short-term and long-term complications. Nevertheless, insulin is fairly effective, albeit a cumbersome and imperfect therapy. Our group has long espoused the need to use combination approaches much like those that have proven effective in treating cancer or HIV (25). Still, we must recall that type 1 diabetes is neither cancer nor HIV. Potential combination approaches, including those that include cell therapy, should be associated with appropriately low risk profiles, especially when being considered for use in children.

The potential of umbilical cord blood to participate in the future of type 1 diabetes interventional therapies exists. Nevertheless, multiple therapeutic avenues will need to be explored, and several modalities will likely need to be combined to achieve the dream of safely and permanently reversing or preventing type 1 diabetes. Future efforts to use autologous umbilical cord blood in the treatment of type 1 diabetes will continue with emphasis on mechanistic studies, establishment of age-appropriate comparative groups, and addition of multiple safe therapies (i.e., vitamin D and n-3 fatty acids) in hopes of achieving synergy.

Acknowledgments

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No potential conflicts of interest relevant to this article were reported.

The sponsors of the study had no role in the study design, data collection, data analysis, interpretation of data, or writing of the report.

We acknowledge the assistance of Hilla-Lee Viener (laboratory technician), Douglas Theriaque (data manager), the stem cell laboratory staff and nurses, the General Clinical Research Center staff and nurses, and, most importantly, the children and families who participated in this phase I trial.

Footnotes

Clinical trial reg. no. NCT00305344, clinicaltrials.gov.

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See accompanying editorial, p. 2138.

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Autologous Umbilical Cord Blood Infusion for Type 1 Diabetes

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Abstract

Objective—The physical, emotional, and economic costs of type 1 diabetes (T1D) mandate continued efforts to develop effective strategies to prevent or reverse the disease. Herein, we describe the scientific and therapeutic rationale underlying efforts utilizing umbilical cord blood (UCB) as a therapy for ameliorating the progression of this autoimmune disease.

Patients and Methods—We recently embarked on a pilot study to document the safety and potential efficacy of autologous UCB infusion in subjects with T1D. Under this protocol, patients recently diagnosed with the disease and for whom autologous cord blood is stored, undergo infusion. Studies are performed before infusion and every 3–6 months post-infusion for immunologic and metabolic assessment. To date 15 autologous infusions have been performed.

Results—Preliminary observations suggest that autologous cord blood transfusion is safe and provides some slowing of the loss of endogenous insulin production in children with T1D. Mechanistic studies demonstrate that umbilical cord blood contains highly functional populations of regulatory T cells (Treg) and that increased Treg populations may be found in the peripheral blood of subjects more than 6 months after cord blood infusion. We provide the rationale for cord blood based therapies, a summary of our initial protocol, and plans for future studies designed to explore the potential of cord blood derived regulatory T cells to treat T1D.

Conclusions—Prolonged follow up and additional mechanistic efforts are urgently needed to determine if umbilical cord blood derived stem cells can be used as part of safe and effective therapies for T1D.

Keywords

Type 1 Diabetes; Cord Blood; Stem Cells; Islets

Introduction - The Need to Prevent or Reverse Type 1 Diabetes

Type 1 diabetes (T1D) is an autoimmune disease characterized by T-cell mediated destruction of insulin producing beta cells and lifelong dependence on exogenous insulin administration. T1D affects nearly 1 in 300 within the United States and the incidence of the disease continues to rise at approximately 3% per year [1,2]. On an international level, the incidence of T1D varies dramatically; as much as 500-fold [3].

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Since the discovery of insulin by Banting, Best, Collip, and McCleod in 1921, T1D has evolved from a uniformly fatal disease to a chronic disease; one with a continuously evolving armamentarium of devices and insulin analogs that have greatly improved care. Nevertheless, the overwhelming majority of patients with T1D are still unable or unwilling to utilize currently available resources that would minimize their risk of diabetes related complications [4]. With this, the tremendous physical, emotional, and economic costs of T1D demand that we explore novel strategies for the prevention and reversal of T1D.

Over the last 25 years, a majority of efforts seeking to ameliorate the autoimmune process and reverse disease in those recently diagnosed have focused on the use of immunosuppressive or immunomodulatory drugs [5,6]. While several of these agents have shown and continue to show promise (e.g., anti-CD3), no single agent has succeeded in demonstrating long term success in preventing or reversing T1D. More recently, additional efforts have focused on the use of both autologous and allogeneic stem cells as sources of new islets, and perhaps more intriguingly, as potential sources of safe and effective immunomodulation [7–9]. Among the broad array of potential cell based therapies, the use of autologous umbilical cord blood (UCB) as a source of immunomodulatory cells for the treatment of autoimmune diseases has become an increasingly popular concept [10–13]; this, based on the potential for UCB to potentially restore proper immune regulation.

Type 1 Diabetes - A Disease Characterized by Loss of Tolerance

The pathophysiology of T1D in both humans and non-obese diabetic (NOD) mice appears largely related to an innate defect in the immune system culminating in a loss of self tolerance and destruction of the insulin producing beta cells [14,15]. NOD mice, the prototypic animal model for human T1D, have obvious defects in central and peripheral tolerance [16] and exhibit a variety of abnormalities in immune function (e.g., reduced IL-2 production, proliferative hyporesponsiveness, etc.)[17]. In terms of the cellular basis for this immunoregulatory failure, it is key to note (albeit somewhat controversial) that both NOD mice and patients with T1D have potential deficiencies in at least two T cell populations intimately involved in immune regulation; NKT cells and CD4+CD25+ or so called "regulatory" T cells (Treg) [18–22]. In addition to defects in T cell based immune regulation, developmental and functional defects have also been reported in B-lymphocytes as well as antigen presenting cells (APC) of both NOD mice and human's with T1D; including those of differentiation and function of macrophages and DC [23–29].

Because of this, an intense degree of research interest has recently been generated towards an improved understanding of the mechanisms that regulate the immune response and form a state of immunological tolerance. Indeed, the ability to develop a means for imparting tolerance may have a dramatic impact not only on the way autoimmune disorders such as T1D are treated, but in addition, towards therapeutic applications related to allergy, transplantation, and oncology.

Why Stem Cell Therapy in Type 1 Diabetes?

Currently, the only means allowing for the reversal of T1D involves whole pancreas or islet cell transplantation in association with non-specific immunosuppressive therapy [30]. Unfortunately, immunosuppressive therapy not only results in compromised immune function, but the potential exists for additional complications with the long-term use of this form of intervention. This facet, combined with limitations afforded by the paucity of available cadaveric organs, have resulted in ever increasing attention towards the potential use of embryonic stem cells, cord blood derived stem cells, adult stem cells, pancreatic progenitor cells, and the transdifferentiation of other non-pancreatic cells as a means to cure this disease [31].

Indeed, recent advances in stem cell research provide an exciting and potentially new approach towards finding a cure for T1D and many other clinical disorders. Stem cells possess the capacity to multiply and to differentiate into a variety of cell populations. As evidence of the potential for these cells to treat T1D, bone marrow transplantation has been shown to prevent autoimmune insulitis and diabetes in NOD mice [32]. In addition, recent research in immunodeficient mice with chemically-induced pancreatic damage has shown that bone marrow-derived stem cells may have the capacity to initiate beta cell regeneration [33]. However the mechanism involved in pancreatic regeneration may be somewhat contrary to the classic concept of direct stem cell differentiation into cells of the desired target tissue. In their chemically induced diabetes model, Hess et al. determined that transplanted bone marrow derived stem cells travel preferentially to damaged organs and initiate tissue regeneration via the organ's own stem cell population [33]. As such, it may be that the role of stem cells in ameliorating T1D is to protect remaining beta cells from further destruction or stimulate remaining tissue to regenerate rather than participating directly in the production of de-novo stem cell derived islets. The question remains, do similar processes occur at the level of the human pancreas?

Autologous Transplantation in Humans with Type 1 Diabetes

In terms of human application, autologous stem cell transplantation, in which the transplant recipient is the stem cell donor, is the most common and potentially safest form of stem cell transplantation. Autologous bone marrow transplants have been used successfully for patients undergoing high dose chemotherapy, and for the treatment of many forms of cancer [34]. More recently, stem cell transplants have also been used as a treatment option for autoimmune disorders including multiple sclerosis (MS), Evans syndrome, lupus, and rheumatic disorders [35–37]. While initial attempts at using transplantation to treat autoimmune diseases involved traditional myeloablative protocols, non-myeloablative or "lymphoablative" protocols have recently demonstrated remarkable success in treating autoimmune disease. Non-myeloablative approaches are clearly less risky than traditional myeloablative regimens. Nevertheless, the risk of serious morbidity or even mortality with non-myeloablative transplants may still be unacceptably high. In perhaps the largest series of non-myeloblative transplants for autoimmune disease performed to date (180 patients) the overall mortality rate is approximately 1.7% [38]. As mortality was only observed in patients with longstanding, severe autoimmune diseases treated for many years with immunosuppressive regimens, the question remains what the morbidity and mortality rates would be in otherwise healthy subjects with new onset autoimmune disease?

Voltarelli et al recently published the first attempts to determine the safety and efficacy of non-myeloablative autologous transplantation in new onset T1D patients [13]. In this study of 15 new onset T1D subjects (mean age 19.2 years) undergoing non-myeloablative auto-transplantation, subjects underwent autologous stem cell mobilization with cyclophosphamide ($2g/m^2$) and daily granulocyte colony stimulating factor (10μg/kg/d) followed by leukapheresis and cryopreservation of stem cells. Following conditioning with rabbit anti-thymocyte globlulin (4.5mg/kg) and cyclophosphamide (200mg/kg), the previously mobilized cells were re-infused intravenously. Fourteen of the 15 subjects were able to discontinue insulin injections for at least one month post therapy, with the majority being able to remain off insulin for over 6 months. Fortunately, no mortality was observed in their small study. Nevertheless, morbidity was still an issue. Male patients had semen samples stored prior to treatment to preserve fertility, all patients received antimicrobial and antifungal prophylaxis, mean hospital stay was 19.2 days (range 15–24), and nearly all patients experienced the common transplantation-related complications of febrile neutropenia, nausea, vomiting, and alopecia.

These results force us to continue the discussion as to what level of risk physicians, patients, and parents of children with T1D are or should be willing to take to achieve a potential cure. While T1D is undoubtedly a terrible life long disease, the success of contemporary treatment modalities make the use of any therapy with a significant mortality risk unacceptable. Whether a mortality risk of 1%, 0.1%, or even 0.01% is justifiable for a potential cure is a difficult question to answer but one we must grapple with as we explore new therapies. As such, we remain cautiously optimistic that stem cell therapies can be safely modified and applied to the treatment of T1D. Because our priority is to develop effective strategies that minimize and preferably eliminate the risk for treatment related severe morbidity or mortality, our group's recent focus has been the potential use of non-pretreated autologous UCB transfusion in children with T1D.

Why Cord Blood? - Properties and Therapeutic Potential

In an era where the mere mention of "stem cell therapies" stirs controversy, the use of UCB is attractive as it avoids much of the debate surrounding this delicate issue. In addition, UCB offers additional major advantages over other ethically acceptable stem cell sources. When compared to bone marrow and peripherally mobilized stem cells, UCB is preferable because of its immediate availability, absence of risk to the donor (and if autologous to the recipient as well) low risk of graft-versus-host disease, and increased capacity for expansion [39]. As such, UCB has been used successfully in transplantation for a variety of diseases, including acute lymphocytic and myeloid leukemia, lymphoma, Fanconi anemia, and sickle cell disease [34]. While the use of UCB transplantation has been hampered somewhat by the relatively small number of stored cord blood samples, the fixed number of cells available in single UCB unit, and the overall lack of experience with UCB transplantation, UCB transplantation could replace bone marrow transplantation as the standard of care in the near future [40].

From a research perspective, UCB has shown great promise as a source for deriving HLA matched hematopoietic stem cell populations. The ability of UCB-derived stem cells to differentiate into a variety of non-blood cell types, including hepatocytes, neural cells, and endothelial cells has already been documented [41]. As further evidence of the potential use of UCB in T1D therapies, UCB stem cells have successfully been directed in vitro to differentiate into insulin and c-peptide producing cells [42].

Since cord blood contains a large population of immature unprimed highly functional regulatory T lymphocytes, this may be the most important reason or exploring therapeutic applications of UCB in T1D. The population of highly functional regulatory T cells in UCB may function to decrease the inflammatory cytokine response and anergize the effector T cells which play a key role in the cellular-mediated autoimmune process [39,43]. As protocols for expanding Treg from UCB continue to evolve, the limitations of cord blood as a limited resource begin to diminish and the therapeutic potential of UCB Tregs continues to expand [44,45]. As such, the role of the cord blood Treg has become the focus of our work in designing UCB based therapies for T1D (FIGURE 1).

Practically, the lack of disease reversal trials for children with T1D under the age of 8 years of age (due to safety concerns with the immunosuppressive regimens being tested) also makes the use of UCB particularly appealing. As the rates of UCB storage continue to increase exponentially, the number of potential subjects for autologous UCB based clinical trials will continue to grow. The fact that UCB is stored at birth without the need for any additional intervention (i.e. bone marrow biopsy or stem cell mobilization and aphaeresis) is an additional advantage in considering an UCB based therapy for children. As UCB storage facilities continue to rethink storage methods that would allow for multiple potential "withdrawals", potential exists for protocols that involve cell expansion or multiple cell infusions.

Autologous Cord Blood Infusion in T1D

Based on available pre-clinical data and the agreement that infusion of minimally manipulated autologous cord blood cells was likely to be extremely safe, we began the process of designing and implementing an unblinded observational pilot study to determine if autologous UCB infusion could ameliorate the T1D autoimmune process and provide patients with preservation of remaining endogenous insulin production.

We hypothesized that autologous UCB transfusion in the setting of T1D may help mitigate the autoimmune process by a number of potential mechanisms: (1) UCB stem cells may migrate to the damaged pancreas where they will differentiate into insulin producing beta cells, (2) UCB stem cells may act as nurse cells to foster the proliferation of new islets from remaining viable tissue, and/or (3) UCB regulatory T cells may facilitate direct or bystander suppression of effector T cells or allow for the restoration of tolerance by their inhibitory effects on multiple cell types [46].

The study has been designed as a 2 year, unblinded, observational study with peak c-peptide following a standard mixed meal tolerance test, HbA1c, and daily insulin requirement set as the primary outcome variables. Subjects undergo mixed meal tolerance testing immediately before cord blood infusion and then every 3 months during the 1st post infusion year and every 6 months in the 2nd post infusion year (FIGURE 2). In addition to measures of insulin production and metabolic control, we study the immunological effects of autologous cord blood infusion by obtaining blood for peripheral blood mononuclear cell (PBMC) analysis at each visit. PBMCs are analyzed by flow cytometry with staining for the cell surface markers CD3, CD4, CD8, CD25, and the intracellular marker FOXP3. In addition, suppression assays are performed to measure the function of peripherally collected Tregs.

Our study has been designed with broad inclusion criteria as little preclinical data are available to guide selection of specific subjects. As such, we allow any child over 1 year of age with T1D, stored autologous cord blood, normal screening labs (complete blood count and basic metabolic profile), and no other significant past medical history to participate in the study. Similarly, no specific criteria as to length of time since diagnosis or baseline insulin production are included. To be usable, the cord blood cell viability must be at least 50%, and both the unit and the maternal sample at time of collection must be free of any infectious disease markers. After a potential subject is identified and consented, an aliquot of the cord blood unit and the child's blood is shipped to our stem cell lab where infectious disease testing and HLA confirmation are performed. Once these screening tests are confirmed, the cord blood unit is shipped to our stem cell lab for storage and the child is scheduled for admission to our general clinical research center. Upon arrival in our research center, the subject undergoes a standard mixed meal tolerance test and has blood drawn for baseline metabolic and immunologic studies. The cord blood unit is then thawed and washed per the standard operating procedures of our stem cell laboratory. An aliquot of the cells is analyzed for viability at infusion, CD34 percentage, and Treg frequency.

Following the preparation of the unit, the subjects receive pretreatment with diphenhydramine and acetaminophen. No chemotherapy or other preparative therapy is given. The thawed cord blood cells (typically in a volume of less than 100 mL) are then infused through a peripheral IV over 10–20 minutes. Following the infusion, subjects are observed closely for at least 6 hours prior to being discharged home. Subjects then return for follow testing as previously described every 3 months in the first post infusion year and every 6 months in the second post infusion year.

Recruitment for our cord blood studies officially began in late 2005 after an investigational new drug permit was obtained from the federal drug administration. Despite initial concerns

that we would have great difficulty in identifying T1D patients with stored autologous UCB, we were pleasantly surprised with the large number of referrals we received after simply posting our study on the clinicaltrials.gov and Children with Diabetes websites. In just over six months, we were contacted by more than 50 families with eligible children. A large majority of eligible subjects declined to participate due to parental concerns over using up the cord blood. Many parents felt inclined to continue to store the cord blood until a more definitive therapy had been developed. This issue brings up an interesting Catch-22 with regards to the use of cord blood for T1D. If, in fact, the main action of UCB cells in our subjects is the amelioration of the autoimmune process, the use of UCB infusion shortly after diagnosis or during the honeymoon phase of the disease may be much more effective than if the cells are used latter on in the disease process. As such, saving the cells for potential future use may turn out to be less advantageous unless manipulation of the cells can provide for both stem cell mediated islet regeneration and restoration of immune tolerance.

In June of 2007 we reported preliminary data from our first 8 subjects to reach the 6-month post UCB infusion visit [11,12]. At enrollment, the average age of our infused subjects at that time was 5.29 ± 1.8 years (range 2.4–7.3), the average duration of T1D was 0.84 ± 0.8 years, and the average HbA1c was 6.3 ± 0.7%. We compared the daily insulin requirements (units/kg/day) and the HbA1c values of our infused cord blood subjects with an age matched and disease duration matched group of contemporary intensively treated "controls" from our diabetes clinic (n=13, age 4.5 ± 2.2 years, duration of diabetes 0.77 ± 0.6 years). While this comparison has significant flaws in that the control subjects were not part of the initial study and that the study subjects were highly motivated to maintain the best possible control, we did observe significant differences between the groups which suggested benefit in the UCB infusion group [11,12].

In terms of providing evidence of a mechanism for the action of UCB, our group has focused on the frequency and function of Treg in the peripheral blood of our UCB infused subjects. While poor access to the pancreas limits our ability to dissect the importance of the potential mechanisms involved in humans, we hope to employ both our clinical trial data and novel humanized mouse models of T1D to elucidate the potential efficacy and mechanisms involved in UCB therapies for T1D. Undoubtedly, these intriguing data support further efforts to characterize these critical immunoregulatory cells.

Perhaps the most important finding to report at this time is that we had absolutely no significant adverse events associated with the study. As mentioned previously, our group feels that interventional efforts in T1D must continue to put the highest value on patient safety. To date, we have safely infused a total of 15 T1D subjects with autologous UCB. Our FDA approval allows for a total of 23 subjects to be treated under this protocol and as such, we are continuing to recruit eligible subjects. We expect to formally report data once 10 subjects have reached the one year post infusion visit with the final report coming after all subjects have reached the two year post infusion visit. While preliminary data remain supportive of the concept that UCB infusion provides benefit, we would caution readers to patiently await the reporting of more robust data before making conclusions. Though the potential of UCB to participate in the future of T1D interventional therapies is immense, the reality remains that multiple therapeutic avenues will need to be explored and that several modalities will likely need to be combined in order to achieve the dream of safely and permanently reversing or perhaps even preventing T1D.

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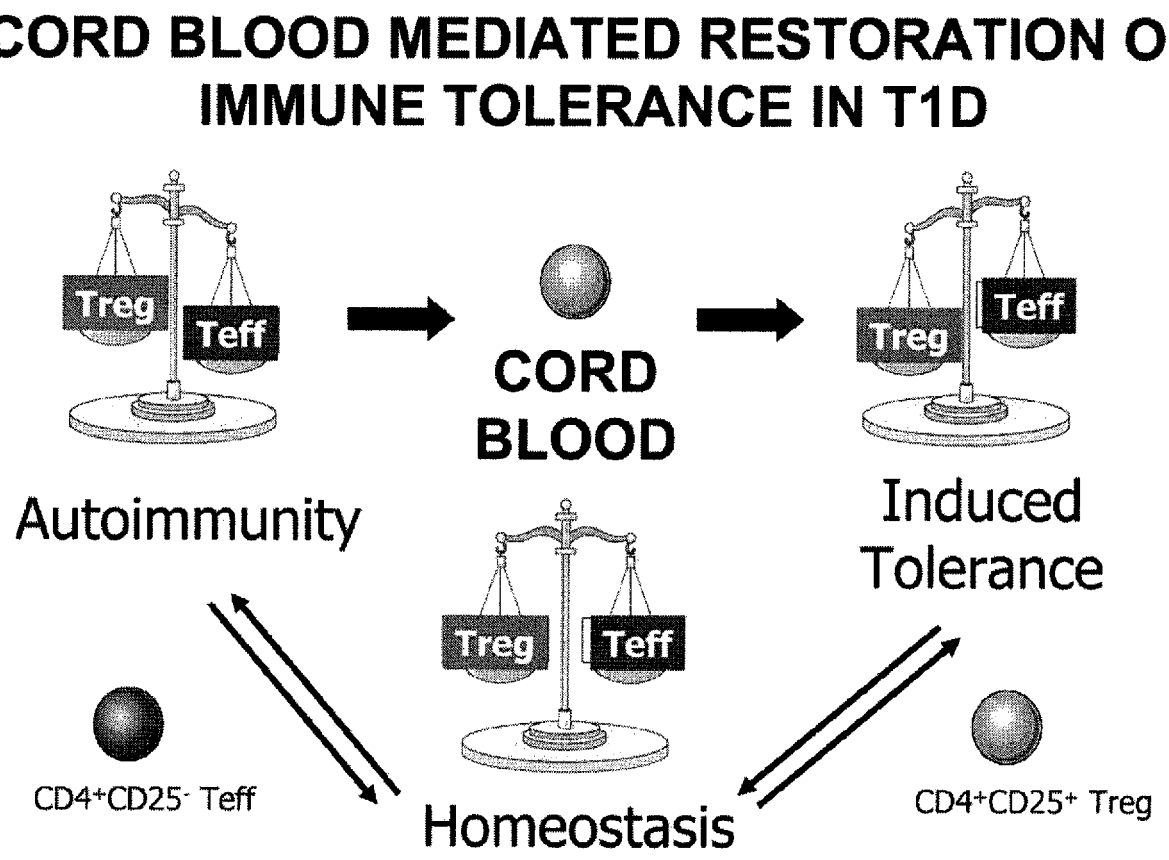

FIGURE 1. Cord Blood Mediated Restoration of Immune Tolerance In T1D
This theoretical model of the basic imbalance between Treg number or function seen in autoimmune disease demonstrates the simple concept that a rich source of Treg such as cord blood may have the potential to tip the scales back in favor or immune tolerance. If true, this concept could be applied the treatment of many autoimmune diseases. (Figure produced, with permission, using software from www.servier.com).

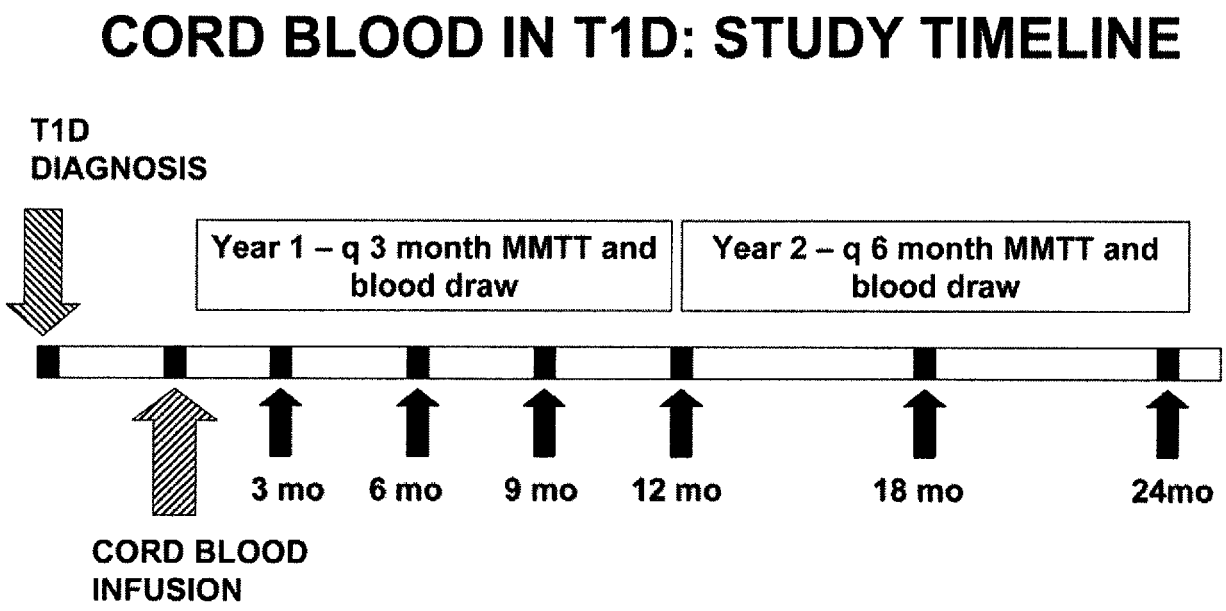

FIGURE 2. Cord Blood in T1D Study Timeline

Our study was designed to be a 2 year observational study of the effects of autologous cord blood infusion in children with T1D. We follow each child every 3 months during the first year post infusion and every 6 months during the second year post infusion. Blood is obtained for metabolic and immunologic studies at each visit.